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U.S. FISH & WILDLIFE SERVICE
REGION 6

CONTAMINANTS PROGRAM

TOXICITY OF SURFACE WATERS FROM BENTON LAKE NATIONAL WILDLIFE REFUGE AND FREEZOUT LAKE WILDLIFE MANAGEMENT AREA, MONTANA, TO MALLARD DUCKLINGS

BY

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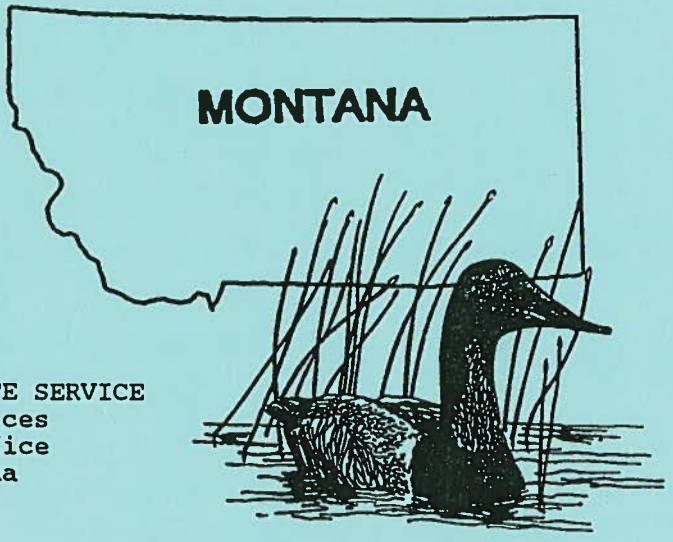

U.S. FISH AND WILDLIFE SERVICE
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ABSTRACT

We measured the growth and survival of captive mallard (Anas platyrhynchos) ducklings housed in wire cages during a 28-day drinking water bioassay to assess potential effects caused by their consumption of surface water. Surface water samples used in the bioassay drinking water treatments were collected from three sites in Benton Lake National Wildlife Refuge (NWR), three sites in Freezout Lake Wildlife Management Area (WMA), and a freshwater spring. Those sites provided a treatment range from low to high salinity and selenium. Mean selenium concentrations in duckling liver tissue at the end of the exposure were less than 4.5 μg/g-dry weight in all treatment groups and not significantly different among groups. These low selenium concentrations provide evidence that selenium uptake via drinking water is not a major pathway for the bioaccumulation of selenium by ducklings.

Ducklings drinking water from a site within Freezout WMA that had a specific conductance of 43,000 μS/cm died during the first 72 hours of exposure. No other drinking water treatments caused significant duckling mortality during the bioassay. From day 7 through the remainder of the bioassay, consumption of water collected from a site in Benton Lake NWR with a specific conductance of 15,500 μS/cm significantly reduced duckling growth compared to the freshwater spring treatment. Drinking water treatments having a specific conductance of 5,920 μS/cm or less did not adversely affect duckling growth. Therefore, exposure to moderately saline water adversely affected duckling growth during the entire 28-day exposure. These findings indicate that measurement of specific conductance in wetland habitats can be used to identify saline surface waters that pose a hazard to duckling health.

INTRODUCTION

Benton Lake NWR and Freezout Lake WMA are located adjacent to the Sun River Irrigation Project in north-central Montana. The wildlife areas consist of freshwater wetland units that are surrounded by upland habitat and are primarily managed to promote waterfowl, shorebird, and upland game bird production.

Orthmeyer and Ball (1990) followed the broods of 31 radio-marked mallards at Benton Lake NWR and observed total brood loss among 37% of the females. Specific causes of brood mortality could not be determined, but environmental contamination may be a contributing factor. Data gathered from 1986 to 1991 within the Sun River Irrigation Project (Knapton et al. 1988, Palawski et al. 1991) indicated that some water sources entering the wildlife areas were relatively saline and had elevated selenium concentrations.

In laboratory experiments, Mitcham and Wobeser (1988a,b) demonstrated that survival of mallard ducklings drinking highly saline water was significantly reduced. Also, environmentally elevated selenium concentrations have been shown to reduce waterfowl growth, survival, and reproductive success in laboratory studies (Heinz et al. 1987) and in the field (Ohlendorf et al. 1986). Selenium is cycled through water and sediment in wetland habitats and bioconcentrated in the aquatic food chain. Waterfowl are potentially exposed

to selenium through drinking water and dietary consumption of wetland food chain organisms that have bioaccumulated selenium (Lemly and Smith 1987). The objectives of this study were to develop a field-oriented bioassay method using mallard ducklings as the test organism and to determine if the survival or growth of ducklings is affected by their drinking of saline surface water collected from Benton Lake NWR and Freezout Lake WMA.

METHODS

We obtained 100 1-day-old mallard ducklings from a commercial game farm (Whistling Wings, Inc., Hanover, Illinois) and randomly assigned them to 16 groups of six ducklings each. Ducklings in two of the 16 groups were sacrificed immediately. For each of these two pre-exposure groups, a composite brain sample was analyzed for sodium and a composite liver sample was analyzed for trace elements by the Environmental Trace Substances Research Center (ETSRC), Columbia, MO. Each of the 14 remaining groups was housed in a wire-and-particle-board cage (0.9 x 0.9 x 0.6 m) equipped with a brood lamp. The groups were provided with commercial (minimum of 22% protein) duckling food ad libitum. Samples of the duckling food were analyzed for trace elements by ETSRC.

Twelve of the duckling groups were subjected to experimental exposures, and two groups were controls. The two control groups were exposed for 28 days to drinking water collected from a freshwater spring (Giant Springs, Great Falls, MT). The twelve experimental groups were exposed to six replicated treatments consisting of drinking water obtained from three sites in Benton Lake NWR and three sites in Freezout Lake WMA. The treatments ranged from low to high salinity and selenium concentrations. We obtained water for the experimental groups from Priest Butte Lakes Seep, the south end of Freezout Lake, Freezout WMA Pond 5, Benton Lake NWR Pool 4A Drainage, Benton Lake NWR Pool 2, and Benton Lake NWR Pool 6 (Figures 1 and 2). We measured specific conductance each time water was collected. Water samples collected from each of those sites on 30 June 1992 and 13 July 1992 were analyzed for selected major ions (chloride, sulfate, sodium, and magnesium) and selenium by ETSRC.

The exposure period to commercial diet and drinking water treatments was 28 days. Duckling survival was monitored every day. Duckling mass, tarsus length, and culmen length were recorded on days 1, 7, 14, 21, and 28. All ducklings found dead during the exposure period were retrieved and their brains and livers removed. Individual brain samples from these ducklings were analyzed for sodium. Liver samples from ducklings that had died during exposure were composited, by group, for selenium analysis by ETSRC. All ducklings remaining alive at the end of the exposure period were sacrificed by asphyxiation with carbon dioxide and their brains and livers removed for individual analysis of sodium in brain tissue and trace elements in liver tissue. Procedures used by ETSRC to analyze selenium and major ions in drinking water, feed, and biological tissues are described by the U.S. Fish and Wildlife Service (1985).

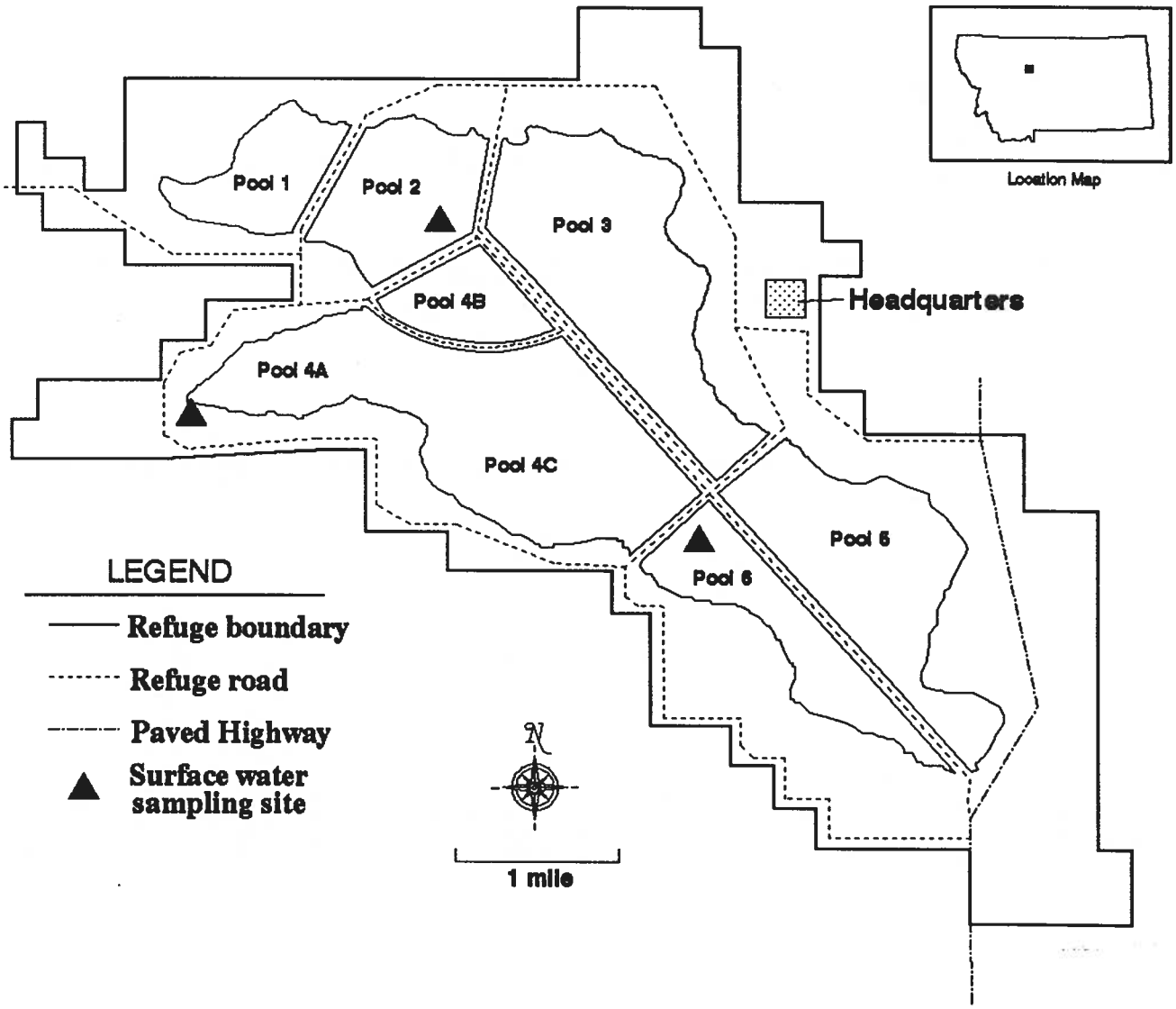

Figure 1. Surface water sites used as drinking water treatments in a 28-day duckling bioassay.

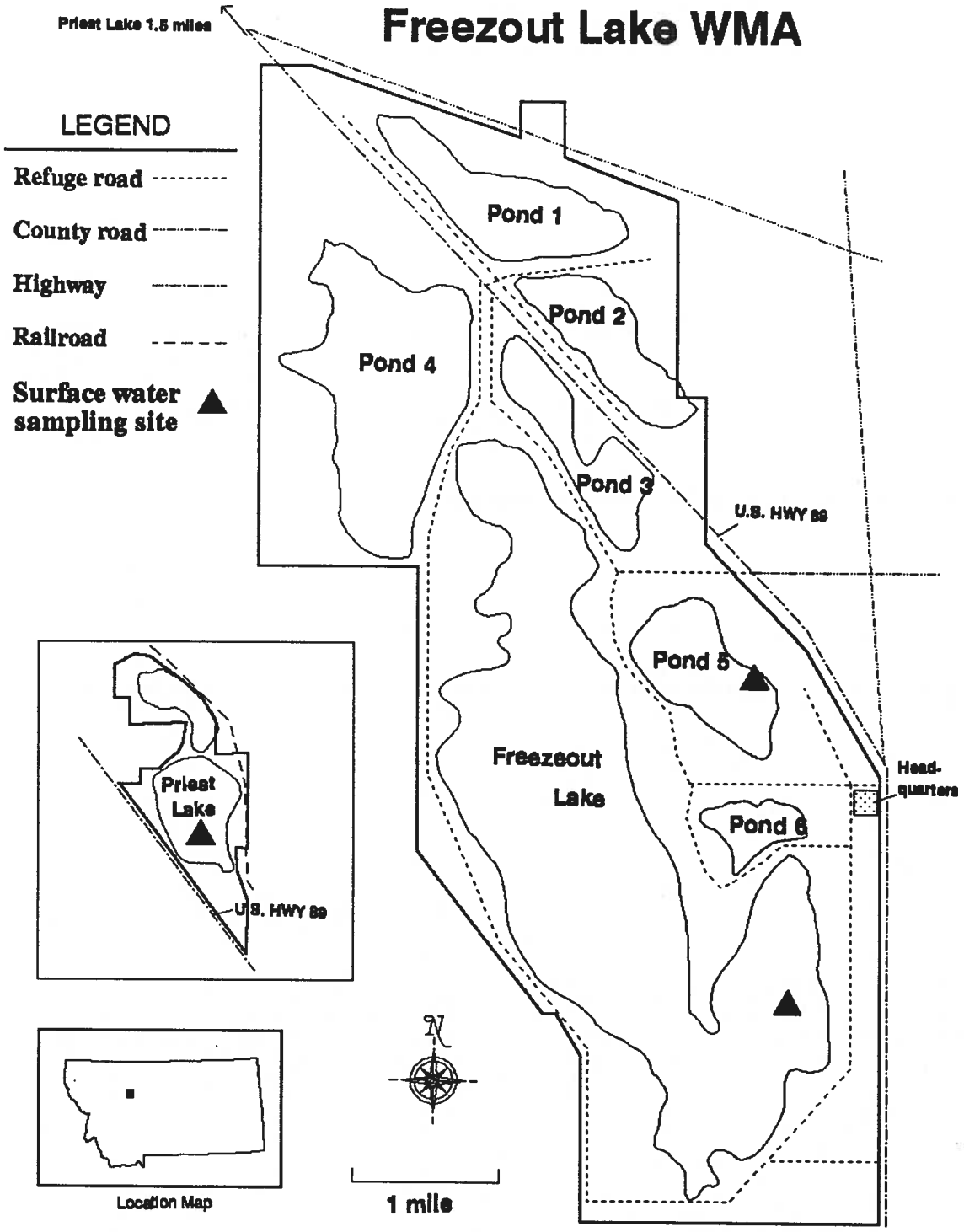

Figure 2. Surface water sites used as drinking water treatments in a 28-day duckling bioassay.

Laboratory quality control of samples analyzed by ETSRC was assured through the Patuxent Analytical Control Facility (PACF), Laurel, MD. The precision and accuracy of laboratory analyses were confirmed with procedural blanks, duplicate analyses, test recoveries of spiked material, and reference material analyses. Round-robin tests among U.S. Fish and Wildlife Service and contract analytical laboratories also were part of the PACF quality assurance review. All contaminant analyses performed as part of this study received a PACF quality assurance review.

We compared measurements of mass, tarsus length, and culmen length for surviving ducklings in each treatment group for each measurement day (days 1, 7, 14, 21, and 28) using the analysis of variance (ANOVA) procedure for nested unbalanced designs provided by SYSTAT (Wilkinson 1987). We used the within-treatment mean square as the error term in all ANOVA procedures. When the ANOVA among groups was found to be significant ($p < 0.05$), we used SYSTAT to conduct post hoc comparisons to identify any significant difference between each treatment group and the control group.

RESULTS AND DISCUSSION

All ducklings in the group receiving water from Priest Butte Lakes Seep died within 72 hours. Six ducklings from this group died during the first 24 hours of exposure and five of the six remaining ducklings died within 48 hours. Two other ducklings died during the exposure period. A duckling from the group receiving water from the south end of Freezout Lake was found dead on the morning of day 1, and another from the control group was found dead on the morning of day 6. When those ducklings were necropsied, the individual from the South Freezout Lake group was found to have sustained a head injury. Given the circumstances associated with these two mortalities, it is unlikely that their deaths were caused by their respective drinking water treatments.

None of the five surviving treatment groups differed significantly (ANOVA, $p > 0.05$) from the control group for measures of body mass, tarsus length, or culmen length on day 1 of the exposure period (Table 1). However, significant differences (ANOVA, $p < 0.05$) were found among the groups on days 7, 14, 21, and 28 for all measures (Tables 1 and 2). Ducklings from the group receiving water from the Benton Lake Pool 4A Drainage were significantly smaller than the control group for measures of body mass and tarsus length on all measurement days after day 1, and for culmen length on days 7, 14, and 28. Ducklings from the groups receiving water from Freezout Lake Pond 5 and Benton Lake Pool 6 did not differ significantly from the controls in any measure. Ducklings from the groups receiving water from the south end of Freezout Lake and Benton Lake Pool 2 differed significantly from the control group for some measures on days 7, 14, and 21; but, by day 28 only ducklings from the group receiving Benton Lake Pool 4A Drainage water differed significantly from the control group for any measure and differed for all measures.

Liver mass was measured at necropsy on day 28, and only a marginally significant difference was found among groups (ANOVA, $p = 0.053$). Only the liver masses of ducklings receiving water from Benton Lake Pool 4A Drainage were significantly different from the control group ($p < 0.025$).

Table 1. Arithmetic mean body mass, culmen length, and tarsus length of ducklings during the first 21 days of a drinking-water bioassay using surface water from Benton Lake NWR and Freezout Lake WMA. --, No data.

Measurement	Freezout Lake WMA				Benton Lake NWR			Control
	Priest Lakes	Butte Seep	South Lake	Pond 5	Pool 4A Drainage	Pool 6	Pool 2	Giant Springs
Day 1								
Body mass, in g	--		45	45	40	46	44	43
Culmen length, in mm	--		14	13	13	13	13	12
Tarsus length, in mm	--		28	27	28	29	28	27
Day 7								
Body mass, in g	--		172[A]	164	116[A]	137	158	147
Culmen length, in mm	--		23[A]	22	17[A]	20	21	21
Tarsus length, in mm	--		40	39	32[A]	39	39	38
Day 14								
Body mass, in g	--		349[A]	300	207[A]	323	236[A]	316
Culmen length, in mm	--		31	30	26[A]	29	28	29
Tarsus length, in mm	--		51	50	44[A]	50	46[A]	50
Day 21								
Body mass, in g	--		461	435	266[A]	441	369[A]	482
Culmen length, in mm	--		35	34	30	34	33	33
Tarsus length, in mm	--		57	56	50[A]	57	53[A]	56

[A] Significantly different from control (ANOVA, post hoc comparisons $p < 0.05$).

Table 2. Physical and chemical measurements of ducklings on day 28 of a drinking-water bioassay using surface water from Benton Lake NWR and Freezout Lake WMA. Data are presented as arithmetic means.

	Freezout Lake WMA			Benton Lake NWR			Control
Analyte	Priest Lakes	Butte Seep	South Lake	Pond 5	Pool 4A Drainage	Pool 6	Pool 2	Giant Springs
				Day 28 - Necropsy at conclusion of experimental exposure			
Mean specific conductance, in µS/cm at 25°C	43,000	2,630	365	15,500	5,920	693	630
Survival, in %	0[A]	92	100	100	100	100	92
Liver selenium, in ug/g	2.5[A]	4.5	4.3	4.1	3.8	4.0	3.8
Brain sodium, in ug/g	9,730[A]	6,380	6,170	6,340	6,370	6,450	6,570
Body mass, in g	ND[B]	607	533	327[C]	550	485	556
Liver mass, in g	ND	24	22	17[C]	29	21	27
Culmen length, in mm	ND	33	32	28[C]	32	31	33
Tarsus length, in mm	ND	58	58	50[C]	58	55	59

[A] Due to 100% mortality in this treatment, data correspond to 72 hours of exposure.

[B] ND = No Data

[C] Significantly different from control (ANOVA, post hoc comparisons, $p < 0.05$).

Most day 28 morphometric measurements of control treatment ducklings used in this bioassay agreed closely with those reported by Hoffman et al. (1991) for 28-day-old mallards reared on a 22% protein diet. However, "tarsus length" values in this study were much shorter than the "tarsal length" values reported by Hoffman et al. (1991). We used a modification of the tarsus measurement technique described by Dzubin and Cooch (n.d.) as total tarsus length, intended for use on live birds. Our technique may have differed from that employed by Hoffman et al. (1991).

Waterborne selenium concentrations ranged from 0.8 to 530 µg/L among treatment groups (Table 3). Mean selenium concentration in the two feed samples was 0.76 µg/g dry weight. Liver selenium concentrations among surviving groups of ducklings were not significantly different (ANOVA, $p = 0.318$). Mean selenium concentrations for each group (Table 3) were substantially less than the 8.0 µg/g dry weight concentration calculated by J.P. Skorupa (written commun.) to be the mean background concentration of selenium in the livers of ducks (presumably adults) collected from relatively uncontaminated wetlands. The low selenium concentration in livers of these captive mallard ducklings indicates that selenium bioaccumulation in wild ducks utilizing Benton Lake NWR and Freezout Lake WMA occurs primarily through the dietary intake of food chain organisms that have accumulated selenium rather than by consumption of waterborne selenium in their drinking water.

Waterborne sodium concentrations ranged from 6.5 to 2080 mg/L among treatment groups (Table 3). Mean sodium concentrations in water from Priest Butte Lakes Seep and Benton Lake Pool 4A Drainage exceeded the 1,500 mg/L concentration at which decreased feather growth occurred in mallard ducklings (Mitcham and Wobeser 1988a). We found no significant difference among the surviving groups for brain sodium concentration (ANOVA, $p = 0.358$). However, the mean sodium concentration (9,730 µg/g dry weight) in the brains of ducklings that received water from Priest Butte Lakes Seep and died within the first 3 days of exposure was significantly higher than the mean brain sodium concentration (6,650 µg/g dry weight) in the pre-exposure groups sacrificed on day 1 (t-test, $p < 0.001$). The order in which the ducklings drinking Priest Butte Lakes Seep water died was significantly correlated with their brain sodium concentrations (Spearman's $r = 0.672$, $p < 0.02$), suggesting that brain sodium concentrations can be used to diagnose the occurrence of salt toxicosis in ducklings.

Although we do not know the specific cause of death or growth inhibition for affected ducklings, we believe that specific conductance of sample water (Table 3) is an appropriate measure of potential toxic effects. Priest Butte Lakes Seep water, used in the treatment that had a specific conductance of 43,000 µS/cm, was quickly fatal to the ducklings. Water from Benton Lake Pool 4A Drainage, with a mean specific conductance of 15,500 µS/cm, was associated with reduced duckling growth, and Benton Lake Pool 6 water, with the third highest mean specific conductance of 5,920 µS/cm, showed no evident toxic effects.

Table 3. Chemical measurements of drinking water treatments during a 28-day duckling bioassay using surface water from Benton Lake NWR and Freezout Lake WMA. [--, No data.]

Analyte	Freezout Lake WMA			Benton Lake NWR			Control
	Priest Butte Lakes Seep	South Lake	Pond 5	Pool 4A Drainage	Pool 6	Pool 2	Giant Springs
				Water - Day 0			
Specific conductance, in uS/cm	43,000	2,760	294	16,400	7,260	770	631
Chloride, in mg/L	282	70	3.3	734	141	9.8	6.3
Sodium, in mg/L	2,020	290	6.6	2,080	865	59	9.6
Sulfate, in mg/L	56,200	1,300	36	11,500	3,720	191	152
Magnesium, in mg/L	12,300	141	18	1,470	477	54	28
Selenium, in ug/L	530	5.5	0.7	11	0.9	0.8	0.8
				Water - Day 13			
Specific conductance, in uS/cm	--	2,610	327	13,100	6,290	695	--
Chloride, in mg/L	--	60	2.5	826	161	8.8	--
Sodium, in mg/L	--	275	6.5	1,550	791	57	--
Sulfate, in mg/L	--	1,300	44	8,900	3,180	181	--
Magnesium, in mg/L	--	154	20	1,140	412	41	--
Selenium, in ug/L	--	21	1.0	2.2	1.0	0.7	--

Our findings are consistent with those of Mitcham and Wobeser (1988b), who reported that 1-day-old mallard ducklings exposed to natural saline water with a specific conductance of 35,000 µS/cm died within 60 hours, and that those exposed to a specific conductance of 67,000 µS/cm died within 30 hours. Ducklings in their 20,000 µS/cm group experienced a 60% mortality rate after 14 days of exposure. Mitcham and Wobeser (1988a) found that 14-day-old mallard ducklings exposed to water with a specific conductance of 15,250 µS/cm stopped eating, became inactive, and sometimes died.

Swanson et al. (1984) found that mallard ducklings exposed to lake water with a specific conductance of 17,000 µS/cm had significantly lower growth rates than did control ducklings. The mean mass in their treated group was 42% less than the mean mass in their control group after 9 days. We found similar 35% and 41% reductions in body mass in our Benton Lake Pool 4A Drainage group (15,500 µS/cm) compared to controls after 14 and 28 days of exposure, respectively.

Most duckling mortality documented in this study and other studies occurred within the first few days of exposure to highly saline water. Riggert (1977) suggested that the salt gland of a duckling does not become capable of excreting salt until it is 6 days old, although Mitcham and Wobeser (1988a) reported that mallard ducklings as young as 4 days old may develop functional salt glands when exposed to highly saline water. However, exposure to moderately saline water in this study adversely affected duckling growth during the entire 28-day exposure. Our results represent the effects of saline water on ducks at what may be their most susceptible time of life.

MANAGEMENT RECOMMENDATION

In order to maintain Benton Lake NWR and Freezout Lake WMA as premiere wetland/prairie complexes for waterfowl, shorebirds, and the myriad of other water birds that thrive there, efforts should be undertaken to manage salinity levels in wetland units. We recommend that salinity levels, measured as specific conductance, be no greater than 6,000 µS/cm in any of the marsh units, and no more than 5,000 µS/cm for all units combined at each complex when water is at planned management levels in any given year.

WORK EFFORT

Investigators, who might consider using this bioassay technique, should be aware of the time commitment required to conduct this type of study. Not including time spent planning the study, obtaining materials, and constructing the cages, we spent approximately 47 person-days conducting this bioassay. Although the daily feeding, watering, and monitoring of the ducklings took little time, the process of weighing and measuring the ducklings and cleaning their cages each week was very time-consuming. We expended approximately 9 person-days conducting the necropsy of the surviving ducklings at the end of the exposure period and preparing the resulting tissue samples for analysis. A duckling bioassay is much more labor-intensive than the commonly employed aquatic bioassays using small organisms and short exposure periods and will be more expensive to conduct.

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LITERATURE CITED

Dzubin, A., and Cooch, E., n.d., Measurements of geese: general field methods. California Waterfowl Association, Sacramento, California, 20 p.

Heinz, G.H., D.J. Hoffman, A.J. Krynitsky, and D.M.G. Weller. 1987. Reproduction in mallards fed selenium. Environ. Toxicol. Chem. 6:423-433.

Hoffman, D.J., C.J. Sanderson, L.J. LeCaptain, E. Cromartie, and G.W. Pendleton. 1991. Interactive effects of boron, selenium, and dietary protein on survival, growth, and physiology in mallard ducklings. Arch. Environ. Contam. Toxicol. 20:288-294.

Knapton, J.R., W.E. Jones, Jr., and J.W. Sutphin. 1988. Reconnaissance investigation of water quality, bottom sediment, and biota associated with irrigation drainage in the Sun River area, west-central Montana, 1986-87. U.S. Geol. Surv., Water-Resour. Investig. Rep. 87-4244.

Lemly, A.D., and G.J. Smith. 1987. Aquatic cycling of selenium: implications for fish and wildlife. U.S. Fish Wildl. Serv., Fish Wildl. Leaflet No. 12.

Mitcham, S.A., and G. Wobeser. 1988a. Effects of sodium and magnesium sulfate in drinking water on mallard ducklings. J. Wildl. Diseases 24:30-44.

Mitcham, S.A., and G. Wobeser. 1988b. Toxic effects of natural saline waters on mallard ducklings. J. Wildl. Diseases 24:45-50.

Ohlendorf, H.M., R.L. Hothem, C.M. Bunck, T.W. Aldrich, and J.F. Moore. 1986. Relationships between selenium concentrations and avian reproduction. Trans. N. Amer. Wildl. Natur. Resour. Conf. 51:330-342.

Orthmeyer, D.L., and I.J. Ball. 1990. Survival of mallard broods on Benton Lake National Wildlife Refuge in Northcentral Montana. J. Wildl. Manage. 54:62-66.

Palawski, D.U., W.E. Jones, Jr., K. DuBois, and J.C. Malloy. 1991. Contaminant biomonitoring at the Benton Lake National Wildlife Refuge in 1988. U.S. Fish Wildl. Serv., Fish Wildl. Enhancement, Montana State Office, Helena, Montana.

Riggert, T.L. 1977. The biology of the mountain duck on Rottnest Island, Western Australia. Wildl. Monogr. 52. 67 pp.

Swanson, G.A., V.A. Adomaitis, F.B. Lee, J.R. Serie, and J.A. Shoesmith. 1984. Limnological conditions influencing duckling use of saline lakes in south-central North Dakota. J. Wildl. Manage. 48:340-349.

U.S. Fish and Wildlife Service. 1985. Procedures for resource contaminant assessment analytical contract work. Habitat Resources Instructional Memorandum. Washington, D.C. 203 p.

Wilkinson, L. 1987. SYSTAT: The system for statistics: Version 3.0. Evanston, Illinois, SYSTAT, Inc.

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